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Prospects of biodiesel production from microalgae in India

Shakeel A. Khan [a,*], Rashmi [b], Mir Z. Hussain [a], S. Prasad [a], U.C. Banerjee [b]

[a] Division of Environmental Sciences, Indian Agricultural Research Institute, New Delhi 110012, India

[b] Department of Pharmaceutical Technology (Biotechnology), National Institute of Pharmaceutical and Education Research (NIPER), Sector 67, Phase X, S.A.S. Nagar, Mohali 160062, Punjab, India

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ABSTRACT

Energy is essential and vital for development, and the global economy literally runs on energy. The use of fossil fuels as energy is now widely accepted as unsustainable due to depleting resources and also due to the accumulation of greenhouse gases in the environment. Renewable and carbon neutral biodiesel are necessary for environmental and economic sustainability. Biodiesel demand is constantly increasing as the reservoir of fossil fuel are depleting. Unfortunately biodiesel produced from oil crop, waste cooking oil and animal fats are not able to replace fossil fuel. The viability of the first generation biofuels production is however questionable because of the conflict with food supply. Production of biodiesel using microalgae biomass appears to be a viable alternative. The oil productivity of many microalgae exceeds the best producing oil crops. Microalgae are photosynthetic microorganisms which convert sunlight, water and CO_2 to sugars, from which macromolecules, such as lipids and triacylglycerols (TAGs) can be obtained. These TAGs are the promising and sustainable feedstock for biodiesel production. Microalgal biorefinery approach can be used to reduce the cost of making microalgal biodiesel. Microalgal-based carbon sequestration technologies cover the cost of carbon capture and sequestration. The present paper is an attempt to review the potential of microalgal biodiesel in comparison to the agricultural crops and its prospects in India.

Contents

* Corresponding author. Tel.: +91 11 25841490; fax: +91 11 25841866.
E-mail addresses: shakeel_iari@yahoo.com, shakeel_env@iari.res.in (S.A. Khan).

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1. Introduction

The world has been confronted with an energy crisis due to depletion of finite resources of fossil fuel. Continued use of petroleum-based fuels is now widely recognized as unsustainable because of depleting supplies and contribution of these fuels to pollute the environment. India meets nearly 75–80% of its total petroleum requirements through imports. The hydrocarbons sector plays vital role in the economic growth of the country. With the increase in share of hydrocarbons in the energy supply/use, this share of imported energy is expected to exceed 90% by 2030 [1]. The estimated energy supply mix in India for a period up to 2025 is given in Table 1. In ''Hydrocarbons Vision-2025'', the demand for petroleum products has been forecasted on the basis of the estimated GDP growth and the expected oil elasticity. The oil elasticity was estimated based on the past trends and the structural changes occurring in the economy and has been assumed to decrease from the current 1.1 (average over the last few years) to 0.7 in 2025. Oil and gas continues to play a pre-eminent role in meeting the energy requirements of the country and it is estimated that 45% of the total energy needs would be met by the oil and gas sector [1]. The gap between supply and availability of crude oil, petroleum products as well as gas from indigenous sources is likely to increase over the years (Table 2). The challenge, therefore, is to secure adequate energy supplies at the least possible cost.

1.1. World's scenario

Over 1.5 trillion barrels of oil equivalent have been produced since Edwin Drake drilled the world's first oil well in 1859. The world will need that same amount to meet demand in the next 25 years alone [2]. The International Energy Agency (IEA) has reported in the reference scenario that the world's primary energy need is projected to grow by 55% between 2005 and 2030, at an average annual rate of 1.8% per year. Fossil fuels remain the dominant source of primary energy, accounting for 84% of the overall increase in demand between 2005 and 2030 [3]. Energy and capital have reported that, by 2025, the world's demand for oil will shoot up to 60%, while production capacity would be thrown back to 1985 levels. According to the Energy Information Agency (EIA) report, petroleum consumption fell by 90,000 barrels per day in 2008–2009 [3]. China's annual oil consumption growth rate is around 7.5% and while that of India's is 5.5% and both are expected to take quantum leap over the next decade [2]. If the governments around the world stick to current policies, the world will need almost 60% more energy in 2030 than today, of this 45% will be accounted by China and India together. Transportation is one of the fastest growing sectors using 27% of the primary energy [3]. At the present staggering rates of consumption, the world fossil oil reserve will be exhausted in less than 45 years [4].

Table 1
Share of future energy supply in India (%).

Year	Coal	Oil	Gas	Hydel	Nuclear
1997–1998	55	35	7	2	1
2001–2002	50	32	15	2	1
2006–2007	50	32	15	2	1
2010–2011	53	30	14	2	1
2024–2025	50	25	20	2	3

Up to 2011 from Technical Note on Energy, Planning Commission, Govt. of India (1998–1999). Beyond this period the figures have been extrapolated.

Table 2
Demand of petroleum products in Mt (Hydrocarbon Vision-2025).

Year	Demand (without meeting gas deficit)	Demand (with meeting gas deficit)	Estimated refining capacity	Estimated crude requirement
1998–1999	91	103	69	69
2001–2002	111	138	129	122
2006–2007	148	179[a]	167	173
2011–2012	195	195[b]	184	190
2024–2025	368	368	358	364

Report of the sub-group on development of refining, marketing transportation and infrastructure requirements 1999 (MoPNG).
[a] Assuming 15 million metric tonnes per annum (MMTPA) of LNG import by 2007.
[b] Assuming that by 2012, adequate gas is available through imports and domestic sources.

1.2. India's scenario

The increasing import of fuel has necessitated the search for other liquid fuels as an alternative to diesel, which is being used in large quantities in transport of industrial and agricultural sector [5]. According to an estimate, automobiles alone contribute to 70% of the total petroleum consumptions. The country faces problems in regard to the fuel requirement for increased transportation demand [6]. At present the consumption of petroleum products in

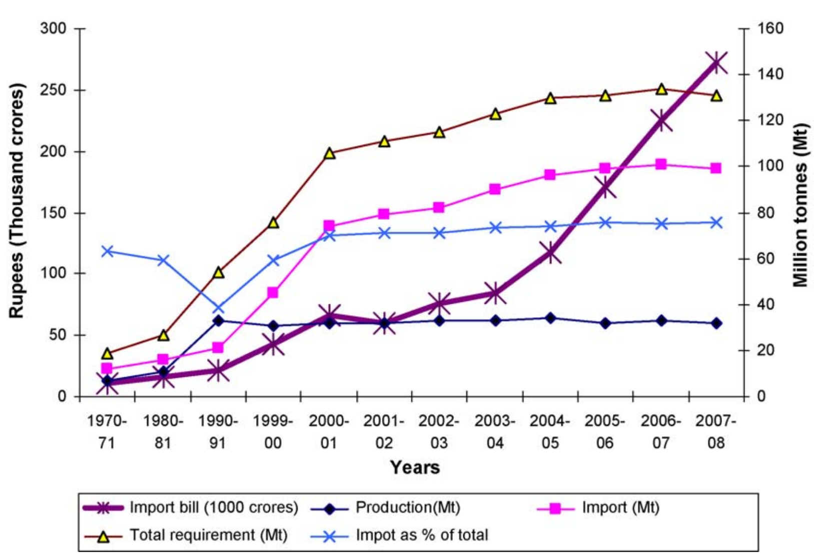
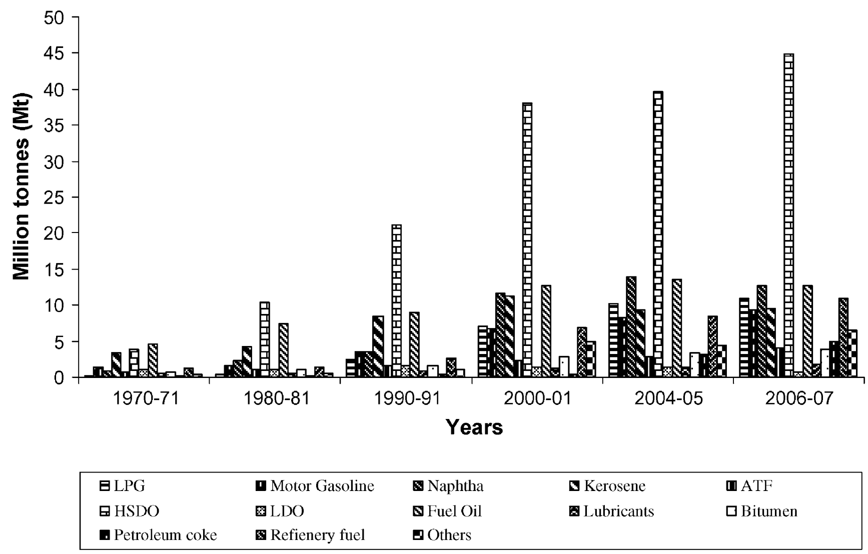


Fig. 1. Scenario of crude oil import and production in India.

India has been increasing at annual growth rate of 5–6% and in the year 2005–2006, it was about 112 million tonnes (Mt)/year [6]. According to annual report (2006–2007) of Ministry of Petroleum and Natural Gas (MoPNG), India has imported about 99 Mt of crude oil during the year 2005–2006, causing a heavy burden of Rs. 171,702 crores on foreign exchange [7]. For the 2007–2008, the crude oil import bill was Rs. 272,699 crores ($68 billion), having more than 75% of oil import dependency (Fig. 1). At the end of 2007, India produced 37.3 Mt of crude oil, i.e. 1% of the world's total crude oil while consumed 128.5 Mt of crude oil, i.e. 3.3% of the total world's consumption. At the end of year 2007, the proved oil reserve of India was 0.7 thousand (Mt) and India's share was about 0.4% of the world's total oil reserves with the reserve-to-production (R/P) ratio of 18.7 years [4]. Proved oil reserve is the quantities that can be recovered in the future from known reservoirs under existing economic and operating conditions. Reserve-to-production (R/P) ratio is the length of time that remaining reserves would last if production continued at current rate.

India's transportation fuel requirements are unique in the world. India consumes almost five times more diesel fuel than gasoline, whereas, almost all other countries in the world use more gasoline than diesel fuel. Diesel burns roughly 64 Mt, or 450 million barrels, a year, as opposed to about 84 million barrels of gasoline [7]. During the last two decades, diesel consumption has increased enormously (Fig. 2). Sector wise (end use) consumption of diesel oil is given in Fig. 3. An overall break-up of the percentage of diesel usage by each sector, for the year 2006–2007 is shown in Fig. 4. Thus, in India, search for alternatives to petrodiesel is of special importance and the use of biodiesel is comparatively much more important for us than for rest of the countries. Due to higher demand for fuel it is expected that crude oil production will start declining from the beginning of 2012. Therefore, alternative biodiesel is the only option to fulfill the requirements in future.



Light distillates : Liquid petroleum gas (LPG), Motor Gasoline, Naphtha

Middle distillates : Kerosene, Aviation turbine fuel (ATF), High speed diesel Oil (HSDO), Light diesel oil (LDO)

Heavy ends : Fuel oil, Lubricants, Bitumen, petroleum coke

Others : Includes those of light distillates, middle distillates and heavy ends and imports through private parties

Fig. 2. Consumption of petroleum products in India.

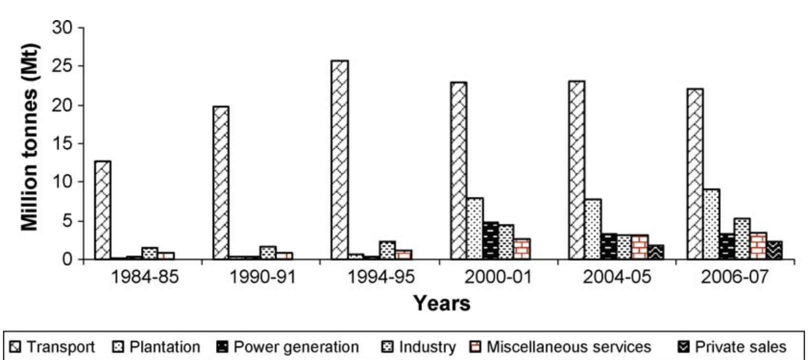


Fig. 3. Sector wise (end use) consumption of diesel oil.

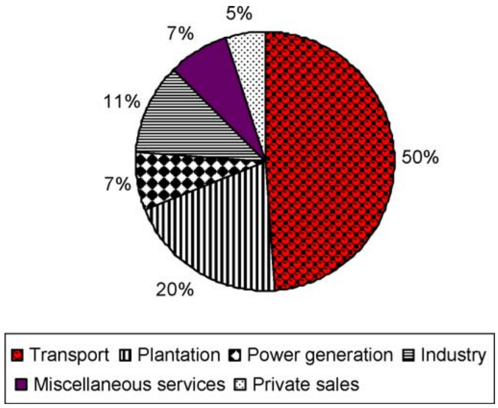


Fig. 4. An overall break-up of the percentage of diesel usage by each sector, for the year 2006–2007.

2. Alternative diesel fuels

There has been greater awareness on alternative diesel fuel in India due to shortage of petrodiesel and soaring prices of crude oil. Significant activities have picked up for its production especially with a view to reduce the huge cost involved in import of petroleum fuel and to take care of the shortage of petrodiesel anticipated within a few years from now. In addition, the process of production of biodiesel from carbon neutral biomass will boost the rural economy and providing non-polluting, biodegradable and safe environment [8]. A number of literatures have shown that triglycerides (TAGs) hold promises as alternative diesel engine fuels [9–14].

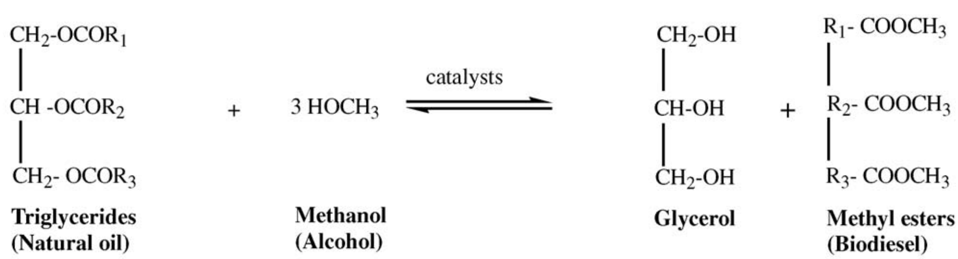
2.1. Biodiesel

Biodiesel is the monoalkyl esters of long-chain fatty acids derived from renewable feedstocks, such as vegetable oil or animal fats [5]. Biodiesel primary advantages are that it is one of the most renewable fuels and also non-toxic and biodegradable [9]. Today, biodiesel has come to mean a very specific chemical modification of natural oils. The use of vegetable oils as alternative fuels has been around for 100 years when the inventor of the diesel engine Rudolph Diesel first tested peanut oil, in his compression ignition engine [11]. Oilseed crops such as rapeseed and soybean oil have been extensively evaluated as sources of biodiesel. One of the biggest advantages of biodiesel compared to many other alternative transportation fuels is that it can be used in existing diesel engines without modification, and can be blended in at any ratio with petroleum diesel.

2.2. Chemical composition of biodiesel and transesterification

The plant oils usually contain free fatty acids, phospholipids, sterols, water, odorants and other impurities. Because of these, the oil cannot be used as fuel directly. To overcome these problems the oil requires slight chemical modification mainly transesterification, pyrolysis and emulsification. Among these, the transesterification is the key and foremost important step to produce the cleaner and environmentally safe fuel from vegetable oils [5]. Natural oil present in the oilseeds generally is in the form of TAGs [10]. TAGs consist of three long chains of fatty acids attached to a glycerol backbone. In 1980s, a chemical modification of natural oils was introduced that helped to bring the viscosity of the oils within the range of current petroleum diesel. By reacting these TAGs with simple alcohols (a chemical reaction known as "transesterification"), a chemical compound known as an alkyl ester is formed, which is known more generically as biodiesel (Fig. 5). The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol [9]. Biodiesel properties are very close to those of petroleum diesel.

Transesterification requires 3 mol of alcohol for each mole of triglyceride to produce 1 mol of glycerol and 3 mol of methyl esters



Where R1, R2, and R3 are long chains of carbons and hydrogen atoms, sometimes called fatty acid chains.

There are five types of fatty acid chains that are common in soybean oil and animal fats (others are present in small amounts).

Fatty acid	No. of carbon : No. of double bond	Fatty acid chain
Palmitic	16:0	R = - $(CH_2)_{14}$ – CH_3
Stearic	18:0	R = - $(CH_2)_{16}$ – CH_3
Oleic	18:1	R = - $(CH_2)_7$ CH=CH $(CH_2)_7CH_3$
Linoleic	18:2	R=-$(CH_2)_7$CH=CH-CH_2-CH=CH $(CH_2)_4CH_3$
Linolenic	18:3	R= - $(CH_2)_7$ CH=CH-CH_2-CH=CH-CH_2-CH=CH-CH_2-CH_3

Fig. 5. Transesterification of triacylglycerols (TAGs).

(Fig. 5). Industrial processes use 6 mol of methanol for each mole of triglyceride [10]. This large excess of methanol ensures that the reaction is driven in the direction of methyl esters, i.e. towards biodiesel. Yield of methyl esters exceeds 98% on a weight basis [10]. Transesterification is catalyzed by acids [10]; alkalis [5] and lipase enzymes [15]. Alkali-catalyzed transesterification is about 4000 times faster than the acid catalyzed reaction [10]. Consequently, alkalis such as sodium and potassium hydroxide are commonly used as commercial catalysts at a concentration of about 1% by weight of oil. Alkoxides such as sodium methoxide are even better catalysts than sodium hydroxide and are being increasingly used. Use of lipases offers important advantages, but is not currently feasible because of the relatively high cost of the catalyst [10]. Alkali-catalyzed transesterification is carried out at approximately 60 °C under atmospheric pressure, as methanol boils at 65 °C at atmospheric pressure. Under these conditions, reaction takes about 90 min to complete. A higher temperature can be used in combination with higher pressure, but this is expensive. Methanol and oil do not mix; hence the reaction mixture contains two liquid phases. Other alcohols can be used, but methanol is the least expensive. To prevent yield loss due to saponification reactions (i.e. soap formation), the oil and alcohol must be dry and the oil should have a minimum of free fatty acids. Biodiesel is recovered by repeated washing with water to remove glycerol and methanol [16].

2.3. Biodiesel is environmental friendly

In view of environmental considerations, biodiesel is considered as 'carbon neutral' because all the carbon dioxide (CO_2) released during consumption had been sequestered from the atmosphere for the growth of vegetable oil crops [8]. Commercial experience with biodiesel has been very promising. The biggest advantages of biodiesel compared to many other alternative transportation fuels is that it can be used in existing diesel engines without modification, and can be blended with petroleum diesel in required ratio. Biodiesel performs as well as petroleum diesel, while reducing emissions of particulate matter, carbon monoxide (CO), hydrocarbons and oxides of sulphur (SOx) [8]. Emissions of oxides of nitrogen (NOx) are, however, higher for biodiesel in many engines. Biodiesel virtually eliminates the notorious black soot emissions associated with diesel engines and the total particulate matter emissions are also much lower. Other environmental benefits of biodiesel include the fact that it is highly biodegradable and appear to reduce emissions of air toxics and carcinogens (relative to petroleum diesel). The environmental benefits of 100% and 20% biodiesel blending, in term of pollutants emission reduction is shown in Table 3. Usage of biodiesel will allow a balance to be sought between agriculture, economic development and the environment.

2.4. Biodiesel development in India

The centerpiece of India's plans for biodiesel development and commercialization was the National Biodiesel Mission (NBM), formulated by the Planning Commission of the Government of India. Based on extensive research carried out in agricultural research centers, it was decided to use *Jatropha curcas* oilseed as the major feedstock for India's biodiesel programme. NBM was planned for two phases. Phase I was termed as demonstration phase and has been carried out from year 2003 to 2007 [17]. The work done during this phase were development of *Jatropha* oilseed nurseries, cultivation of *Jatropha* on 400,000 hectares (ha) waste land, setting up of seed collection and *Jatropha* oil expression centers, and the installation of 80,000 Mt/year transesterification to produce biodiesel from *Jatropha* oil. Phase II planned with a self-sustaining expansion of the programme leading to the production of biodiesel to meet 20% of the country's diesel requirements by 2011–2012.

Table 3
Biodiesel vs. diesel emissions.

Emissions	B100, pure biodiesel	B20, mixed biodiesel (20% biodiesel and 80% petroleum diesel)
Regulated emissions (%)		
Total unburned hydrocarbons	−93	−30
Carbon monoxide	−50	−20
Particulate matter	−30	−22
NOx	+13	+2
Non-regulated emissions (%)		
Sulphates	−100	−20
Polycyclic aromatic hydrocarbons (PAHs)	−80	−13
Nitrated PAHs (NPAHs)	−90	−50
Ozone potential of HC	−50	−10
Life cycle emissions (%)		
Carbon dioxide	−80	
Sulphur dioxide	−100	

Source: Planning Commission of India 2003.
(−): Less % of pollutant emission from biodiesel in comparison to 100% petroleum diesel.
(+): More % of pollutant emission from biodiesel in comparison to 100% petroleum diesel, i.e. only in the case of oxides of nitrogen (NOx).

The lack of assured supplies of vegetable oil feedstock has foiled efforts by the private sector to set up biodiesel plants in India. Commercial biodiesel production has not yet started in India. So far only two firms, Naturol Bioenergy Limited (NBL) and Southern Online Biotechnologies, have embarked on biodiesel projects, both in the southern state of Andhra Pradesh. Naturol Bioenergy Limited (NBL), a joint venture with the Austrian biodiesel firm Energea Gmbh and the investment firm Fe Clean Energy (USA), has planed to install a 300 tonnes/day (t/d)/(90,000 tonnes/year) (t/y) biodiesel plant in Kakinada, Andhra Pradesh [18]. The State Government allocated 120,000 ha of land for *Jatropha* cultivation to the firm but cultivation has not yet begun or is in initial stage. The farmers were demanding that the market set the oilseed price, but NBL wants the government to fix a price to reduce its risks in production. Southern Online Biotechnologies has a 30 t/d (9000 t/y) project, which would require about 9500 t/y of oil. It was expected to get about 6000 t/y through cultivation of *Jatropha* and *Pongamia pinnata* oilseeds on wasteland, and plans to make up the balance through animal fats, but the cultivation of these two cultivar have been failed. So, there are many constraints for the biodiesel production in India and phase I of NBM has not given the anticipated results. Recent research and development on microalgae has exposed a new road ahead that can be promising sustainable feedstock for biodiesel production.

3. Microalgae are emerging feedstock for biodiesel production

Algae are very important from an ecological point of view. Algae are the food source for many animals and belonging to the bottom of the food chain; moreover, they are the principle producers of oxygen on earth. Their important role as food source is due to the content of minerals, vitamins and oils, rich in polyunsaturated fatty acids (PUFAs). PUFAs such as α-linolenic, eicosapentaenoic and docosaesaenoic acids, belongs to the ω-3 group [19–23]. Algae having the ability to synthesize TAGs are considered as a second generation feedstock for production of biofuels, specifically biodiesel. The potential value of microalgal photosynthesis to produce biofuels is widely recognized [15,24–31].

Table 4
The four most important group of algae in terms of abundance.

S. No.	Algae	Known species (near about)	Storage material	Habitat
1	Diatoms (Bacillariophyceae)	100,000	Chyrsolaminarin (polymer of carbohydrates) and TAGs	Oceans, fresh and brackish water
2	Green algae (Chlorophyceae)	8,000	Starch and TAGs	Freshwater
3	Blue-green algae (Cyanophyceae)	2,000	Starch and TAGs	Different habitats
4	Golden algae (Chrysophyceae)	1,000	TAGs and carbohydrates	Freshwater

The advantages of microalgae over higher plants as a source of transportation biofuels are numerous:

1. Microalgae synthesize and accumulate large quantities of neutral lipids/oil [20–50% dry cell weight (DCW)] and grow at high rates (e.g. 1–3 doublings/day).
2. Oil yield per area of microalgae cultures could greatly exceed the yield of best oilseed crops.
3. Microalgae can be cultivated in saline/brackish water/coastal seawater on non-arable land, and do not compete for resources with conventional agriculture.
4. Microalgae tolerate marginal lands (e.g. desert, arid and semi-arid lands) that are not suitable for conventional agriculture.
5. Microalgae utilize nitrogen and phosphorus from a variety of wastewater sources (e.g. agricultural run-off, concentrated animal feed operations, and industrial and municipal wastewaters), providing the additional benefit of wastewater bioremediation.
6. Microalgae sequester CO_2 from flue gases emitted from fossil fuel-fired power plants and other sources, thereby reducing emissions of a major greenhouse gas. 1 kg of algal biomass requiring about 1.8 kg of CO_2 [30].
7. Microalgae produce value-added co-products or by-products (e.g. biopolymers, proteins, polysaccharides, pigments, animal feed and fertilizer) and does not need herbicide and pesticide.
8. Microalgae grow in suitable culture vessels (photobioreactors) throughout the year with higher annual biomass productivity on an area basis.

3.1. Microalgae classification

Photosynthetic organisms that grow in aquatic environments include macroalgae, microalgae and emergents [32]. These are primitive organisms with a simple cellular structure and a large surface to volume body ratio, which gives them the ability to uptake large amount of nutrients. While the mechanism of photosynthesis in microalgae is similar to that of higher plants, they are generally more efficient converters of solar energy because of their simple cellular structure [33–39]. In addition, because the cells grow in aqueous suspension, they have more efficient access to water, CO_2, and other nutrients. For these reasons, microalgae are capable of producing 30 times the amount of oil per unit area of land, compared to terrestrial oilseed crops [16].

Biologists have categorized microalgae in a variety of classes, mainly distinguished by their pigmentation, life cycle and basic cellular structure [32]. The four most important algae (at least in terms of abundance) are shown in Table 4.

3.2. Microalgae as potential source of biofuel

Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass [40]; biodiesel derived from microalgal oil [24,32,34,41] and photobiologically produced biohydrogen [33,35,42–44]. The idea of using microalgae as a source of fuel is not new [35,45,46], but it is now being taken seriously because of the rising price of petroleum and, more significantly, the emerging concern about global warming that is associated with burning of fossil fuels [41].

Recent research initiatives have proven that microalgae biomass appear to be the one of the promising source of renewable biodiesel which is capable of meeting the global demand for transport fuels. Using microalgae to produce biodiesel will not compromise production of food, fodder and other products derived from crops. A comparison of some sources of crop oil is given in Table 5. In view of Table 5, microalgae appear to be the only source of biodiesel that has the potential to completely displace fossil diesel. Oil content in microalgae can exceed 80% by weight of dry biomass [30,47]. Oil levels of 20–50% are quite common (Table 6) [16]. Oil productivity, that is the mass of oil produced per unit volume of the microalgal broth per day, depends on the algal growth rate and the oil content of the biomass. Rodolfi et al. [30] have screened 30 microalgal strains for their lipid production potential by evaluating biomass productivity and lipid content in 250-mL flask laboratory cultures (Table 7). The best lipid producers, that is, the strains showing the best combination of biomass productivity and lipid content, were three members of the marine genus *Nannochloropsis* (out of the six tested), with a lipid content of 30% or higher and a lipid productivity ranging from 55 to

Table 5
Comparison of algae with different crops for biofuel.

Source	Gallons of oil per acre per year
Algae	5000–20,000
Oil palm	635
Coconut	287
Jatropha	207
Rapeseed/Canola	127
Peanut	113
Sunflower	102
Safflower	83
Soybeans	48
Hemp	39
Corn	18

Table 6
Oil content of microalgae (Chisti [16]).

Microalga	Oil content (% dry wt)
Botryococcus braunii	25–75
Chlorella sp.	28–32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16–37
Dunaliella primolecta	23
Isochrysis sp.	25–33
Monallanthus salina	>20
Nannochloris sp.	20–35
Nannochloropsis sp.	31–68
Neochloris oleoabundans	35–54
Nitzschia sp.	45–47
Phaeodactylum tricornutum	20–30
Schizochytrium sp.	50–77
Tetraselmis suecica	15–23
B. braunii	25–75

Table 7
Biomass productivity, lipid content and lipid productivity of 30 microalgal strains cultivated in 250-mL flasks (Rodolfi et al. [30]).

Algal group	Microalgae strains	Habitat	Biomass productivity (g/L/day)	Lipid content (% biomass)	Lipid productivity (mg/L/day)
Diatoms	*Chaetoceros muelleri* F&M-M43	Marine	0.07	33.6	21.8
	Chaetoceros calcitrans CS 178	Marine	0.04	39.8	17.6
	P. tricornutum F&M-M 40	Marine	0.24	18.7	44.8
	Skeletonoma costatum CS 181	Marine	0.08	21.0	17.4
	Skeletonoma sp. CS 252	Marine	0.09	31.8	27.3
	Thalassioria pseudonana CS 173	Marine	0.08	20.6	17.4
	Chlorella sp. F&M-M48	Freshwater	0.23	18.7	42.1
	Chlorella sorokiniana IAM-212	Freshwater	0.23	19.3	44.7
	Chlorella vulgaris CCAP 211/11b	Freshwater	0.17	19.2	32.6
	C. vulgaris F&M-M49	Freshwater	0.20	18.4	36.9
Green algae	*Chlorococcum* sp. UMACC 112	Freshwater	0.28	19.3	53.7
	Scenedemus quadricauda	Freshwater	0.19	18.4	35.1
	Scenedemus F&M-M19	Freshwater	0.21	19.6	40.8
	Scenedemus sp. DM	Freshwater	0.26	21.1	53.9
	T. suecica F&M-M33	Marine	0.32	8.5	27.0
	Tetraselmis sp. F&M-M34	Marine	0.30	14.7	43.4
	T. suecica F&M-M35	Marine	0.28	12.9	36.4
	Ellipsoidion sp. F&M-M31	Marine	0.17	27.4	47.3
	Monodus subterraneus UTEX 151	Freshwater	0.19	16.1	30.4
	Nannochloropsis sp. CS 246	Marine	0.17	29.2	49.7
Eustigmatophytes	*Nannochloropsis* sp. F&M-M26	Marine	0.21	29.6	61.0
	Nannochloropsis sp. F&M-M27	Marine	0.20	24.4	48.2
	Nannochloropsis sp. F&M-M24	Marine	0.18	30.9	54.8
	Nannochloropsis sp. F&M-M29	Marine	0.17	21.6	37.6
	Nannochloropsis sp. F&M-M28	Marine	0.17	35.7	60.9
	Isochrysis sp. (T-ISO) CS 177	Marine	0.17	22.4	37.7
	Isochrysis sp. F&M-M37	Marine	0.14	27.4	37.8
Prymnesiophytes	*Pavlova salina* CS 49	Marine	0.16	30.9	49.4
	Pavlova lutheri CS 182	Marine	0.14	35.5	50.2
Red algae	*Porphyridium cruentum*	Marine	0.37	9.5	34.8

61 mg/L/day). The marine genus *Nannochloropsis* emerged from the screening as one of the best candidates for algal oil production.

3.3. Composition of microalgal oil

Several researchers have reported that algae produced more oil in stressed or unfavourable condition in comparison to optimal growth condition [26]. Under optimal conditions of growth, algae synthesize fatty acids principally for esterification into glycerol-based membrane lipids, which constitute about 5–20% of their DCW. Fatty acids include medium-chain (C10–C14), long-chain (C16–C18) and very-long-chain ($\geq$C20) species and fatty acid derivatives. But under unfavourable environmental or stress conditions, many algae alter their lipid biosynthetic pathways towards the formation and accumulation of neutral lipids (20–50% DCW), mainly in the form of triacylglycerol (TAG). Unlike the glycerolipids found in membranes, TAGs do not perform a structural role but instead serve primarily as a storage form of carbon and energy. After being synthesized, TAGs are deposited in densely packed lipid bodies located in the cytoplasm of the algal cell, although formation and accumulation of lipid bodies also occur in the inter-thylakoid space of the chloroplast in certain green algae [26].

Table 8
Fatty acid composition of microalgal oil (Meng et al. [48]).

Fatty acid	Chain length:no. of double bonds	Oil composition (w/total lipid)
Palmitic acid	16:0	12–1
Palmitoleic acid	16:1	55–7
Stearic acid	18:0	1–2
Oleic acid	18:1	58–60
Linoleic acid	18:2	4–20
Linolenic acid	18:3	14–30

Oil production from microalgae can be performed by both photosynthesis and heterotrophy. The fatty acid composition of typical oil from microalgae is given in Table 8 [48]. It is mainly composed of mixture of unsaturated fatty acids, such as palmitoleic (16:1), oleic (18:1), linoleic (18:2) and linolenic acid (18:3). Saturated fatty acids, palmitic (16:0) and stearic (18:0) are also present to a small extent.

4. Microalgal diesel vs. crop-derived biodiesel

At the present moment, biofuels are derived from food crops such as sugarcane, sugar beet, maize (corn), sorghum, rapeseed, sunflower, soybean and palm, although other forms of biomass can be used, and may be preferable. The most significant concern is the inefficiency and sustainability of these first generation biofuels. In contrast, the second generation biofuels are derived from non-food feedstock. They are extracted from microalgae and other microbial sources, lignocellulosic biomass, rice straw and bioethers, and are a better option for addressing the food and energy security and environmental concerns [31]. There is not enough land space to grow crops for food and feed as well as for biofuel, and to retain the forests and other land uses that sequester carbon in huge quantities. According to one estimate, to replace worldwide petroleum use with biofuel, 10.8 million square miles of farmland with the highest yielding biofuel crops is needed, but unfortunately we have only 5.8 million square miles of farmland on earth. A major criticism often leveled against biomass, particularly against large-scale fuel production, is that it will consume vast swaths of farmland and native habitats, drive up food prices, and result in little reduction in GHG emissions. However, this so-called "food vs. fuel" controversy appears to have been exaggerated in many cases. Credible studies show that with plausible technology developments, biofuels could supply some 30% of global demand in

an environmentally responsible manner without affecting food production [49].

Traditional raw materials for worldwide biodiesel production are mainly coming from four oil crops: rapeseed, sunflower, soybean and palm. In addition, some other oleaginous species such as *J. curcas*, *P. pinnata*, and low value triacylglycerol feedstocks (restaurant grease, animal fat) are being used as an alternative for biodiesel production. Today, biodiesel production is limited and not fully sustainable due to limited availability of raw materials that compete with food and other uses. Based upon the photosynthetic efficiency and growth potential of algae, theoretical calculations indicate that annual oil production of >30,000 l or about 200 barrels of algal oil per hectare of land may be achievable in mass culture of oleaginous algae, which is 100-fold greater than that of soybeans, a major feedstock currently being used for biodiesel in the USA [26]. Timothy et al. [50], argue that a wholesale switch to corn-based ethanol "nearly doubles greenhouse emissions over 30 years and increases greenhouse gases for 167 years", while Fargione et al. [51], reported that converting land to produce food-based biofuels in Brazil, Southeast Asia, and the United States creates a "biofuel carbon debt". New trends look at the second generation of biodiesel where new, no food, feedstock will be used [52]. The most common concern related to the current biofuel systems is that as production capacities increase, so does their competition with agriculture for arable land used for food production [27]. The use of microalgae as energy crops has potential, due to their easy adaptability to growth conditions, the possibility of growing either in fresh or marine waters and avoiding the use of land.

4.1. Road ahead for India

Rapid increases in the price of fossil fuels over the last few years have brought microalgae as alternative biofuel source back into the research and development (R&D) limelight. Literature shows and many researchers recommended the biodiesel as alternative fuels from vegetable oils [8,20,32,34–37]. However in a country like India, which is second most populated country of the world, have to meet the cereal demand first. There is need to explore the possibilities of producing biodiesel from microalgae, which will not compete with the land and cereal crops. In India, about 26 million ha lands are used for the oilseed cultivation, mainly on marginal lands, dependent on monsoon rains (unirrigated) and with low levels of input usage. Yields of oilseeds cultivation are rather low at less than 1 t/ha [53]. With steady growth in population and personal income, Indian per capita consumption of edible oil is also growing steadily. Although, Indian vegetable oil economy is world's fourth largest after USA, China and Brazil. However, oilseeds output and in turn, vegetable oil production have been trailing consumption growth, necessitating imports to meet supply shortfall [53]. Therefore, for country like us, which already depend on the imports of vegetable oil, we have to see the other alternatives for biodiesel production. Since the demand for edible vegetable oil exceeds supply, the government decided to use non-edible oil from *J. curcas* oilseeds as biodiesel feedstock. But the main problem in getting the biodiesel programme rolling is the difficulty linked to initiating large-scale cultivation of *Jatropha*. Farmers do not yet consider *Jatropha* cultivation remunerative enough because the fruits appear after 3 years of crop plantation. For instance, sugarcane plantations yield 70 t/ha and fetch the farmer Rs. 70,000/ha at a sugarcane price of Rs. 1000/t. In comparison, the *Jatropha* farmer gets Rs. 5000/t of oilseeds and if the yield is 3.75 t/ha, his income will be only Rs. 18,750/ha [18]. The other main issue is the lack of seed collection and oil extraction infrastructure. In the absence of this infrastructure and available oilseeds, it will be difficult to persuade entrepreneurs to install transesterification plants. So, whether being edible oil or non-edible oil, we are not getting the viable alternative of biodiesel.

There is an urgent need to design integrated energy farms that are capable of producing fuel and fertilizers besides foods and feed. These energy farms should be established on waste/barren lands and should need least resources like fresh water or chemical fertilizers. Today, the potential value of microalgal photosynthesis to produce biodiesel is, however, widely recognized. So, the road ahead for India is to see the viable option of algal farming.

4.2. Food vs. fuel

Across the world a debate started whether diversion of farmland for biofuel led to the rally and shortage in food grains. This year World Bank has reported that 33 countries may face unrest because of surging food costs and deepening poverty. UN's Food and Agriculture Organization (FAO) has also reported that global food stocks are at their lowest since the 1980s. Many countries had raised the issue of how farmland was being increasingly used to produce biofuel in the developed world leading to a shortfall in grain production. Citing concerns about rising global grain prices, as well as potential land-grabs by large energy firms, a group of Indian ministers has quietly shelved the NBM. (The Economic Times, a leading business newspaper of India has reported on 4th August 2008.)

Carbon neutral renewable liquid fuels are needed to eventually totally displace petroleum-derived transport fuels that contribute to global warming. Biodiesel from oil crops and bioethanol from sugarcane are being produced in increasing amounts as renewable biofuels, but their production in large quantities is not sustainable. An alternative is offered by microalgae. Agricultural oil crops, such as soybean and oil palm, are widely being used to produce biodiesel; however, they produce oils in amounts that are miniscule. As a consequence, oil crops can provide only small quantities of biodiesel for blending with petroleum diesel at a level of a few percent, but they are incapable of providing the large quantities of biodiesel that are necessary to eventually displace all petroleum-sourced transport fuels.

4.3. Microalgae vs. Jatropha

India requires nearly 200 billion gallons of biodiesel annually at the current rate of consumption, if all petroleum-derived transport fuel is to be replaced with biodiesel. To produce this quantity of biodiesel from *Jatropha* oil, *Jatropha* would need to be grown over an area of 952 million acres (384 million ha) [54]. This is more than 100% of all geographic area of the India. Growing *Jatropha* at this scale would, therefore, be totally unrealistic, because no land would be left for producing food, fodder and other crops. Based on these calculations, it is obvious that edible or non-edible crops would not be able to replace petroleum-derived liquid fuels in the foreseeable future. This scenario will however be different if microalgae are used as a source of biodiesel.

An average annual productivity of microalgal biomass in a well designed production system located in a tropical zone ($\pm 37^\circ$ of equator) can be in the region of 1.535 kg/m^3/day [16]. At this level of biomass productivity, and if an average oil content of 30% dry weight in the biomass is assumed, oil yield per hectare of total land area is near about 123 m^3 for 90% of the calendar year. (About 10% of the year is unproductive, because the production facility must be shut down for routine maintenance and cleaning.) This amounts to a microalgal biodiesel yield of 98.4 m^3/ha. Therefore, producing 200 billion gallon of biodiesel that India needs as transport fuel, would require microalgae to be grown over an area of 13 million acres (5.4 million ha) or only 2% of the India geographical area. This is a feasible scenario even if the algal biomass contains only 15% oil by dry

weight. No other potential sources of biodiesel come close to microalgae in being realistic production vehicles for biodiesel.

5. Microalgal biomass production

Conventional open pond algal production systems are age old systems for biomass production. Recently closed photobioreactors have been developed for continuous and increased biomass production. The vast bulk of microalgae cultivated today are grown in open ponds. Open ponds can be built and operated very economically and hence offer many advantages as long as the species for cultivation can be maintained [55]. Producing microalgal biomass is generally more costly than growing crops. To minimize expense, biodiesel production must rely on freely available sunlight, despite daily and seasonal variations in light levels which are easily available in India. In open pond method, fresh culture medium is fed at a constant rate and the same quantity of microalgal broth is withdrawn continuously. Feeding ceases during the night, but the mixing of broth continue to prevent settling of the biomass [56]. There are three potential and very common methods of large-scale production of microalgae

5.1. Open ponds/raceway ponds

Open ponds have a variety of shapes and sizes but the most commonly used design is the raceway pond. It is a closed loop of rectangular grid with recirculation channel. They usually operate at water depths of 15–20 cm, as at these depths biomass concentrations of 1 g dry weight/L and productivities of 60–100 mg/L/day can be obtained [57]. There is a paddlewheel, which mix and circulate the algal biomass as shown in Fig. 6. Flow is guided around bends by baffles positioned in the flow channel. Raceway channels are built in concrete or compacted earth, it can be of different length and diameter and generally lined with white plastic. During daylight, the culture is fed continuously in front of the paddlewheel where the flow begins. Broth is harvested behind the paddlewheel, on completion of the circulation loop. The paddlewheel operates all the time to prevent sedimentation. The main disadvantage of open systems is that by being open to the atmosphere, they loose water by evaporation at a rate similar to land crops and are also susceptible to contamination by unwanted species. In practice open ponds are usually reported to be dominated by two to six species with a range of evolutionary

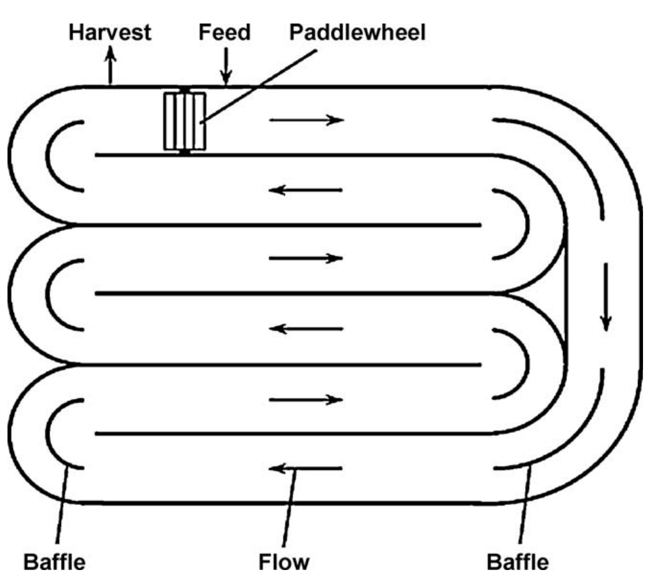


Fig. 6. A raceway pond (reprinted with permission from Chisti [16]).

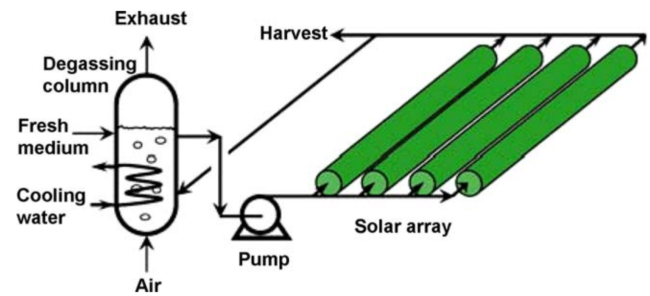


Fig. 7. A tubular photobioreactor with parallel run horizontal tubes (reprinted with permission from Chisti [16]).

advantages; rapid growth, resistance to predators, tolerance to high levels of dissolved oxygen, etc. [27].

Raceways are perceived to be less expensive than photobioreactors, because they cost less to build and operate. Economically, open pond system of biomass production is 10 times less costly in comparison to photobioreactors [32]. Although raceways are of low-cost, they have a low biomass productivity compared with photobioreactors. But in a country like India, there is no need of photobioreactors because its climate is tropical, i.e. $\pm 37^{\circ}$ of equator. Microalgae can be cultivated in the plains and coastal areas of India. There is need to approve the raceway pond system of biomass culture to achieve high and sustained growth rates and oil yields that is essential to developing an algal-based biofuel industry.

5.2. Closed bioreactors

Closed bioreactors support up to fivefold higher productivity with respect to reactor volume and consequently have a smaller "footprint" on a yield basis. Besides saving water, energy and chemicals, closed bioreactors have many other advantages which are increasingly making them the reactor of choice for biofuel production, as their costs are reduced [27]. Closed bioreactors permit essentially single-species culture of microalgae for prolonged durations. Most closed bioreactors are designed as tubular reactors, plate reactors, or bubble column reactors [55,57]. Other less common designs like semi-hollow-spheres have been reported to run successfully [58].

The most common type of closed bioreactor is tubular photobioreactor. Tubular photobioreactor consists of an array of straight transparent tubes that are usually made of plastic or glass. The solar collector tubes are generally 0.1 m diameter or less in diameter because light does not penetrate too deeply in the dense culture broth that is necessary for ensuring a high biomass productivity of the photobioreactor. Microalgal broth is circulated from a reservoir (i.e. the degassing column in Fig. 7.) to the solar collector and back to the reservoir [16].

5.3. Hybrid systems

In hybrid systems, both open ponds as well as closed bioreactor system are used in combination to get better results. Open ponds are a very proficient and lucrative method of cultivating algae, but they become contaminated with superfluous species very quickly. A combination of both systems is probably the most logical choice for cost-effective cultivation of high yielding strains for biofuels. Open ponds are inoculated with a desired strain that was invariably cultivated in a bioreactor, whether it be as simple as a plastic bag or a high tech fiber optic bioreactor. Importantly, the size of the inoculums needs to be large enough for the desired species to establish in the open system before an unwanted species. Therefore to minimize contamination issues, cleaning or flushing the ponds should be part of the aquaculture routine, and

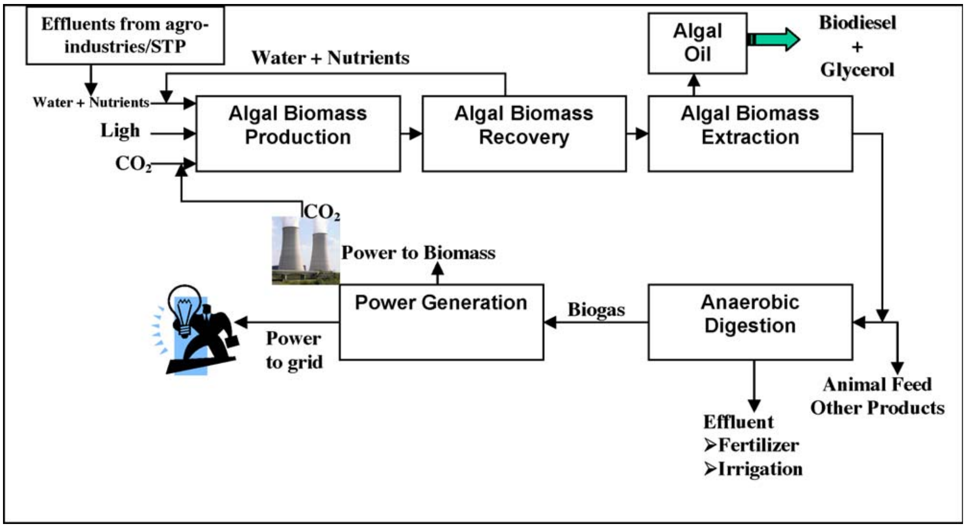


Fig. 8. A conceptual process (biorefinery-based) for producing microalgal biodiesel for better economy.

as such, open ponds can be considered as batch cultures. This process has been demonstrated by Aquasearch (Hawaii, USA) cultivating *Haematococcus pluvialis* for the production of astaxanthin. Half of the Aquasearch facility was devoted to photobioreactors and half to open ponds. *H. pluvialis* is grown continuously in photobioreactors under nutrient sufficient conditions and then a portion is transferred to nutrient-limited open ponds to induce astaxanthin production [27].

6. Algal biorefinery-based production

Like a petroleum refinery, a biorefinery uses every component of the biomass material to produce useable products. Integrated biorefineries are already being operated in Canada, United States, and Germany for producing biofuels and other products from crops such as corn and soybean [16]. This approach can be used to reduce the cost of making microalgal biodiesel. A conceptual process (biorefinery-based) for producing microalgal biodiesel for better economy is given in Fig. 8.

In addition to oils, microalgal biomass contains significant quantities of proteins, carbohydrates and other nutrients [59]. Therefore, the residual biomass from biodiesel production processes can be used potentially as animal and fisheries feed and after anaerobic digestion can be used as fertilizers in the form of compost. The algal dry biomass composition contains up to 46% carbon (C), 10% nitrogen (N) and 1% phosphates (P) and 1 kg of dry algal biomass utilizes up to 1.7 kg CO_2 [26]. So, the microalgal biodiesel projects can qualify as Clean Development Mechanism (CDM) projects and bring in additional income through the sale of Certified Emission Reductions (CER).

6.1. Climate change and CO_2 biomitigation

Global climate change requires immediate and substantial reductions in anthropogenic Green House Gas (GHG) emissions, particularly fossil CO_2. Carbon sequestration could be a major tool for reducing atmospheric CO_2 emissions from fossil fuel usage. Biological CO_2 mitigation has attracted much attention as an alternative strategy because it leads to the production of biomass energy in the process of CO_2 fixation through photosynthesis [60–62]. Biological CO_2 mitigation can be carried out by plants and photosynthetic microorganisms. However, the potential for increased CO_2 capture in agriculture by plants has been estimated to contribute only 3–6% of fossil fuel emissions [63], largely due to the slow growth rates of conventional terrestrial plants. On the other hand, microalgae, a group of fast-growing unicellular or simple multicellular microorganisms, have the ability to fix CO_2 while capturing solar energy with efficiency 10–50 times greater than that of terrestrial plants [64] and produced biomass for the subsequent production of biofuels.

On a worldwide basis, coal is, by far, the largest fossil energy resource available. To put this in perspective, consider the fact that, at current rates of consumption, coal reserves could last for over 200 years. Inevitably, the demand for electricity will have to be met by coal. Coal will remain the mainstay of world baseline electricity generation, accounting for half of electricity generation by the year 2015 [3]. India has vast coal resources, but most are of low quality. Indigenous oil and gas reserves are in short supply while demand for oil almost quadrupled from 1980 to 2005. Oil imports are projected to increase even more going forward, leaving the country more vulnerable to international price spikes and potentially unreliable supplies. In 2005 India ranked fourth in energy consumption, after the United States, China, and Russia. By 2030, India is expected to surpass Russia and be the third largest energy consumer. India has an installed base of about 124,287 MW of electricity as of the year 2006, which includes about 66% thermal energy (85% of which is coal-based) followed by hydro with 26%, nuclear with 3%, and renewable energy with 5% of the current total installed renewable energy base [3]. According to World Energy outlook report of 2007 [3], India's total CO_2 emission in 2007–2008 was 1200 Mt, out of which 800 Mt CO_2 (67%) came from coal combustion. The long-term demand for coal brings with it a demand for technologies that can mitigate the environmental problems associated with coal. While control technologies are used to reduce air pollutants associated with acid rain, no technologies exist today, which address the problem of greenhouse gas emissions. Coal is the most carbon-intensive of the fossil fuels. In other words, for every unit of energy liberated by combustion, coal emits more CO_2 than either petroleum or natural gas. Flue gases from power plant are responsible for more than 7% of the total world CO_2 emissions [65]. CO_2 in flue gas is available at little or no cost. As estimated by the Intergovernmental Panel on Climate Change (IPCC) criteria, the CO_2 concentration of flue gas is up to 15% [28]. Coal is the dominant fuel in India's energy mix, a condition that is expected to persist for at least the next 25 years. We could solve the problem of this huge carbon emission from thermal power plant with the help of algal farm by recycling the carbon. The simple, direct method of GHG mitigation is the

Table 9
Microalgal strains studied for CO_2 biomitigation (Wang et al. [66]).

Microalga	CO_2 (%)	Temp. (°C)	P (g/L/day)	P_{CO_2} (g/L/day)	References
Chlorococcum littorale	40	30	NA	1.0	[67,29]
Chlorella kessleri	18	30	0.087	0.163[a]	[68]
Chlorella sp. *UK001*	15	35	NA	>1	[29]
C. vulgaris	15	–	NA	0.624	[69]
C. vulgaris	Air	25	0.040	0.075[a]	[70]
C. vulgaris	Air	25	0.024	0.045[a]	[70]
Chlorella sp.	40	42	NA	1.0	[65]
Dunaliella	3	27	0.17	0.313[a]	[71]
Haematococcus pluvialis	16–34	20	0.076	0.143	[72]
Scenedesmus obliquus	Air	–	0.009	0.016	[73]
S. obliquus	Air	–	0.016	0.031	[73]
B. braunii	–	25–30	1.1	>1.0	[29]
S. obliquus	18	30	0.14	0.26	[62]
[b]*Spirulina* sp.	12	30	0.22	0.413[a]	[62]

NA = not available.
[a] Calculated from the biomass productivity according to equation, CO_2 fixation rate (P_{CO_2}) $= 1.88 \times$ biomass productivity (P), which is derived from the typical molecular formula of microalgal biomass, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti [16]).
[b] All species except *Spirulina* sp., which is a prokaryotic cyanobacteria (Cyanophyceae) species, are eukaryotic green algae (Chlorophyta) species (Bold and Wynne [74]).

removal of CO_2 from stack gases, followed by long-term sequestration of CO_2 by microalgae ponds. Thus, microalgae technology can extend the useful energy we get from coal combustion and reduce carbon emissions by recycling waste CO_2 from power plants into clean-burning biodiesel. Table 9 summarizes a few microalgal strains that have been studied for CO_2 biomitigation. Some of these strains can tolerate high temperature and CO_2 in the gas stream.

Microalgal-based carbon sequestration technologies can, in principle, not only cover the cost of carbon capture and sequestration but also produce environment friendly biodiesel. Carbon sequestration offers an opportunity for reducing greenhouse gas emission that can complement the current strategies of improving the energy efficiency and increasing the user of non-fossil energy resources.

7. Conclusion

Biodiesel production from microalgae is a goal that still needs much research. There is need for strenuous research on the biosynthesis of algal lipids, especially TAGs, if we want to understand and manipulate algae for the production of biodiesel. While algae appear to provide the natural raw material in the form of a lipid-rich feedstock, our understanding of the details of lipid metabolism to enable manipulation of the process physiologically and genetically is lacking. Over 20 years ago, the Aquatic Species Programme (ASP) of the US Department of Energy (DOE) illustrated the potential of algae to provide liquid energy. To harvest the benefits of that potential will not only require critical engineering innovations and breakthroughs related to algal mass culture and downstream processing, but also focused research on essential biological questions related to regulation of lipid metabolism. Several biological and processing challenges and opportunities lie ahead. Available biochemical knowledge about fatty acid and TAG synthetic pathways in algae is still fragmentary. A critical evaluation of the relationship between the cell cycle and TAG production is needed. Genetic and metabolic engineering are likely to have the greatest impact on improving the economics of production of microalgal diesel. So, there is a need to do the metabolic engineering through genetic manipulation for enhancing the TAG production. Harvest is considered to be an expensive and problematic part of industrial production of microalgal biomass due to low cell density. There is no single best method for harvesting microalgae and reducing their water content. In existing algal aquaculture the most common harvesting processes are flocculation, microscreening and centrifugation. Most importantly, cost-effective and energy-efficient harvesting methods are required to make the whole biofuels production process economical. The concept of coupling a coal-fired power plant with an algae farm provides an elegant approach to recycle of the CO_2 from coal combustion into a useable liquid fuel. Combination of wastewater treatment and microalgal CO_2 fixation provides additional economic incentives due to the savings from chemicals (the nutrients) and the environment benefits. It provides a pathway for removing nitrogen, phosphorus, and metal from wastewater, and producing algal biomass, which can further be exploited for biofuel production, without using freshwater. Use of the biorefinery concept and advances in raceway ponds engineering will further lower the cost of production. If the microalgal biorefinery concept can be adapted to a country like ours, it could become a highly distributed source of fuel oil and perhaps make us self-reliant and improve our economy many folds. In present scenario algal biomass is a key link between energy, local environment and climate change and further research are necessary to unlock full potential of algae.

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